

Please amend the specification as follows:

Please replace the paragraph beginning at page 2, line 7, with the following paragraph:

Although the ATP-dependent ligases and NAD⁺-dependent ligases share little sequence homology, all the ligases investigated so far use the same KXDG (SEQ. ID. No. 24) motif to form adenylated enzyme intermediate (Tomkinson, et al., Bioessays, 19(10):893-901 (1997), Shuman, et al., Virology, 211(1):73-83 (1995), and Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996)). Furthermore, they seem to be organized by similar domains and structural folds ((Doherty, et al., Nucleic Acids Res, 24(12):2281-2287 (1996), Subramanya, et al., Cell, 85(4):607-615 (1996), and Sekiguchi, et al., Nucleic Acids Res, 25(4):727-734 (1997)). The diversity of ligase sequences is not only reflected by their different optimal reaction conditions and kinetic rates, but more importantly by their different specificities toward match and mismatch substrates. Among the viral ATP-dependent ligases, the broad substrate tolerance is represented by the T4 enzyme which seals various mismatches on both the 3' and 5' side of the nick junction (Wu, et al., Gene, 76(2):245-254 (1989)). Vaccinia ligase ligates various mismatches at both 3'-hydroxyl or 5'-phosphate sides with the exception of purine-purine mismatch pairs at the 3'-hydroxyl side (Shuman, S., Biochemistry, 34(49):16138-161475 (1995)). Mammalian ATP-dependent ligases show different substrate sensitivity, as ligase I is more sensitive to 3' mismatches than ligase III (Husain, et al., J Biol Chem, 270(16):9683-9690 (1995)). Additionally, both ligase I and III tolerate a 3'C/T mismatch more than a 3'G/T mismatch. Little is known about archeal ATP-dependent ligases which may reveal the nature of the progenitor of ATP-dependent ligases. Studies on NAD⁺-dependent DNA ligase from *E. coli*, along with T4 ligase, have contributed immensely to understanding of the basic biochemical pathway of the DNA ligation reaction (Lehman, I.R., Science, 186(4166):790-797 (1974) and Rossi, et al., Nucleic Acids Res, 25(11):2106-2113 (1997)). Studies on the NAD⁺-dependent ligase from *Thermus thermophilus* HB8 have revealed the highly discriminative power this enzyme possesses (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Although mismatches at 5'-phosphate side are tolerated to some degree (5'A/C, 5'A/A, 5'C/A, 5'C/T, 5'G/T, 5'G/A, 5'T/T, 5'T/G), mismatches at the 3'-hydroxyl side essentially abolish nick-closure activity except 3'G/T or 3'T/G mismatch (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Apparently, sequence divergence and subsequent subtle structural variation among DNA ligases underlie an enzyme's recognition preferences toward different mismatched base-pairs.

Please replace the paragraph beginning at page 7, line 13, with the following paragraph:

Figures 1A-C show a sequence comparison of *Thermus* DNA ligases.

Figure 1A illustrates the evolutionary tree for *Thermus* DNA ligases. Figure 1B is a regional sequence alignment of nine *Thermus* ligases: *Tsp.* AK16D (SEQ. ID. No. 22); *Thermus aquaticus* YT-1 (SEQ. ID. No. 15); *Thermus Thermophilus* ("Tth") (SEQ. ID. No. 23); *Thermus flavus* (SEQ. ID. No. 16); *Thermus filiformis* Tok4A2 (SEQ. ID. No. 17); *Thermus filiformis* Tok6A1 (SEQ. ID. No. 18); *Tsp.* SM32 (SEQ. ID. No. 19); *Tsp.* Vil3 (SEQ. ID. No. 20); *T. scot* (SEQ. ID. No. 21). The aa (i.e. amino acid) sequence of ~~T. scot~~ *T. scot* is retrieved from ~~Genebank~~ GenBank by accession number 1085749 (SEQ. ID. No. 31), is as follows:

Met	Thr	Leu	Glu	Glu	Ala	Arg	Lys	Arg	Val	Asn	Glu	Leu	Arg	Asp	Leu	1	5	10	15
Ile	Arg	Tyr	His	Asn	Tyr	Arg	Tyr	Tyr	Val	Leu	Ala	Asp	Pro	Glu	Ile	20	25	30	
Ser	Asp	Ala	Glu	Tyr	Asp	Arg	Leu	Leu	Arg	Glu	Leu	Lys	Glu	Leu	Glu	35	40	45	
Glu	Arg	Phe	Pro	Glu	Leu	Lys	Ser	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	50	55	60	
Gly	Ala	Lys	Pro	Leu	Glu	Ala	Thr	Phe	Arg	Pro	Ile	Arg	His	Pro	Thr	65	70	75	80
Arg	Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe	Asn	Phe	Asp	Glu	Leu	Lys	Ala	85	90	95	
Phe	Glu	Glu	Arg	Ile	Gly	Arg	Ala	Leu	Gly	Arg	Glu	Gly	Pro	Phe	Ala	100	105	110	
Tyr	Thr	Val	Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	115	120	125	
Glu	Asp	Gly	Val	Leu	Val	Trp	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Val	130	135	140	
Gly	Glu	Glu	Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	145	150	155	160
Arg	Val	Lys	Gly	Val	Pro	Glu	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	165	170	175	

Met	Pro	Ile	Glu	Ala	Phe	Leu	Arg	Leu	Asn	Glu	Glu	Leu	Glu	Glu	Lys	180	185	190
Gly	Glu	Lys	Ile	Phe	Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	195	200	205
Arg	Gln	Lys	Asp	Pro	Arg	Ile	Thr	Ala	Arg	Arg	Gly	Leu	Arg	Ala	Thr	210	215	220
Phe	Tyr	Ala	Leu	Gly	Leu	Gly	Leu	Glu	Glu	Ser	Gly	Leu	Lys	Thr	Gln	225	230	235
Leu	Asp	Leu	Leu	His	Trp	Leu	Arg	Glu	Lys	Gly	Phe	Pro	Val	Glu	His	245	250	255
Gly	Phe	Ala	Arg	Ala	Glu	Gly	Ala	Glu	Gly	Val	Glu	Arg	Ile	Tyr	Gln	260	265	270
Gly	Trp	Leu	Lys	Glu	Arg	Arg	Ser	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	275	280	285
Val	Val	Lys	Leu	Asp	Glu	Leu	Ser	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	290	295	300
Ala	Arg	Ala	Pro	Arg	Phe	Ala	Ile	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	305	310	315
Lys	Glu	Thr	Arg	Leu	Leu	Gln	Val	Val	Phe	Gln	Val	Gly	Arg	Thr	Gly	325	330	335
Arg	Val	Thr	Pro	Val	Gly	Ile	Leu	Glu	Pro	Val	Phe	Ile	Glu	Gly	Ser	340	345	350
Val	Val	Ser	Arg	Val	Thr	Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu	Glu	Leu	355	360	365
Asp	Val	Arg	Ile	Gly	Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly	Gly	Val	370	375	380
Ile	Pro	Glu	Val	Leu	Arg	Val	Leu	Lys	Glu	Lys	Arg	Thr	Gly	Glu	Glu	385	390	395
Arg	Pro	Ile	Arg	Trp	Pro	Glu	Thr	Cys	Pro	Glu	Cys	Gly	His	Arg	Leu	405	410	415
Val	Lys	Glu	Gly	Lys	Val	His	Arg	Cys	Pro	Asn	Pro	Leu	Cys	Pro	Ala	420	425	430
Lys	Arg	Phe	Glu	Ala	Ile	Arg	His	Tyr	Ala	Ser	Arg	Lys	Ala	Met	Asp	435	440	445
Ile	Gly	Gly	Leu	Gly	Glu	Lys	Leu	Ile	Glu	Lys	Leu	Leu	Glu	Lys	Gly	450	455	460

Pro	Glu	Leu	Lys	Ser	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly	Ala	Arg	
1				5					10					15		
Pro	Leu	Glu	Ser	Thr	Phe	Arg	Pro	Val	Arg	His	Pro	Thr	Arg	Met	Tyr	
			20					25					30			
Ser	Leu	Asp	Asn	Ala	Phe	Ser	Leu	Asp	Glu	Val	Arg	Ala	Phe	Glu	Glu	
		35					40					45				
Arg	Ile	Glu	Arg	Ala	Leu	Gly	Arg	Lys	Gly	Pro	Phe	Leu	Tyr	Thr	Val	
	50					55					60					
Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu	Glu	Gly	
65					70					75					80	
Ile	Leu	Val	Phe	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Thr	Gly	Glu	Glu	
				85					90					95		
Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Arg	Thr	Ile	Pro	Arg	Arg	Leu	Thr	
			100					105					110			
Gly	Val	Pro	Asp	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met	Pro	Ile	
		115					120					125				
Glu	Ala	Phe	Leu	Arg	Leu	Asn	Gln	Glu	Leu	Glu	Glu	Ala	Gly	Glu	Arg	
	130					135					140					
Ile	Phe	Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg	Gln	Lys	
145					150					155					160	
Asp	Pro	Arg	Val	Thr	Ala	Arg	Arg	Gly	Leu	Arg	Ala	Thr	Phe	Tyr	Ala	
			165						170					175		
Leu	Gly	Leu	Gly	Leu	Glu	Glu	Thr	Gly	Leu	Lys	Ser	Gln	His	Asp	Leu	
		180						185					190			
Leu	Leu	Trp	Leu	Lys	Glu	Arg	Gly	Phe	Pro	Val	Glu	His	Gly	Phe	Thr	
		195					200						205			
Arg	Ala	Leu	Gly	Ala	Glu	Gly	Val	Glu	Glu	Val	Tyr	Gln	Ala	Trp	Leu	
	210					215					220					
Lys	Glu	Arg	Arg	Lys	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	Val	Val	Lys	
225					230					235					240	
Leu	Asp	Asp	Leu	Ala	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	Ala	Arg	Ala	
			245						250					255		
Pro	Arg	Phe	Ala	Leu	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Lys	Glu	Thr	
			260					265					270			
Arg	Leu	Leu	Ser	Val	Ala	Phe	Gln	Val	Gly	Arg	Thr	Gly	Arg	Ile	Thr	
		275					280					285				

Pro Val Gly Val Leu Glu	Pro Val Phe Ile Glu Gly	Ser Glu Val Ser
290	295	300
Arg Val Thr Leu His Asn Glu Ser Phe Ile Glu Glu Leu Asp Val Arg		
305	310	315 320
Ile Gly Asp Trp Val Leu Val His Lys Ala Gly Gly Val Ile Pro Glu		
	325 330	335
Val Leu Arg Val Leu Lys Glu Arg Arg Thr Gly Glu Glu Lys Pro Ile		
	340 345	350
Leu Trp Pro Glu Asn Cys Pro Glu Cys Gly His Ala Leu Leu Lys Glu		
	355 360	365
Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe		
	370 375	380
Glu Ala Ile Arg His Tyr Ala Ser Arg Lys Ala Met Asp Ile Gln Gly		
385	390	395 400
Leu Gly Glu Lys Leu Ile Glu Lys Leu Leu Glu Lys Gly Leu Val Arg		
	405 410	415
Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys Glu Asp Leu Leu Asp Leu		
	420 425	430
Glu Arg Met Gly Glu Lys Ser Ala Glu Asn Leu Leu Arg Gln Ile Glu		
	435 440	445
Glu Ser Lys Gly Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu		
	450 455	460
Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu Ala Leu Arg Phe Gly		
465	470	475 480
His Met Asp Arg Leu Leu Glu Ala Gly Leu Gly Asp Leu Leu Glu Val		
	485 490	495
Glu Gly Val Gly Glu Leu Thr Ala Arg Ala Ile Leu Asn Thr Leu Lys		
	500 505	510
Asp Pro Glu Phe Arg Asp Leu Val Arg Arg Leu Lys Glu Ala Gly		
	515 520	525 ;

AF092864 for *Thermus flavus* (SEQ. ID. No. 26), as follows:

Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Glu Gln Val Gly
1 5 10 15
Ala Arg Pro Leu Glu Ala Thr Phe Arg Pro Val Arg His Pro Thr Arg
20 25 30

Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe	Asn	Phe	Asp	Glu	Leu	Lys	Ala	Phe				
35				40				45											
Glu	Glu	Arg	Ile	Glu	Arg	Ala	Leu	Gly	Arg	Glu	Gly	Pro	Phe	Ala	Tyr				
50				55				60											
Thr	Val	Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu				
65				70				75				80							
Asp	Gly	Val	Leu	Val	Tyr	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Val	Gly				
85				90				95											
Glu	Glu	Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg				
100				105				110											
Leu	Lys	Gly	Val	Pro	Glu	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met				
115				120				125											
Pro	Val	Glu	Ala	Phe	Leu	Arg	Leu	Asn	Glu	Glu	Leu	Glu	Glu	Arg	Gly				
130				135				140											
Ala	Arg	Ile	Phe	Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg				
145				150				155				160							
Gln	Lys	Asp	Pro	Arg	Ile	Thr	Ala	Lys	Arg	Gly	Leu	Arg	Ala	Thr	Phe				
165				170				175											
Tyr	Ala	Leu	Gly	Leu	Gly	Leu	Glu	Glu	Val	Glu	Arg	Glu	Gly	Val	Ala				
180				185				190											
Thr	Gln	Phe	Ala	Leu	Leu	His	Trp	Leu	Lys	Glu	Lys	Ser	Phe	Pro	Val				
195				200				205											
Glu	His	Gly	Tyr	Ala	Arg	Ala	Val	Gly	Ala	Glu	Gly	Val	Glu	Ala	Val				
210				215				220											
Tyr	Gln	Asp	Trp	Leu	Lys	Lys	Arg	Arg	Ala	Leu	Pro	Phe	Glu	Ala	Asp				
225				230				235				240							
Gly	Val	Val	Val	Lys	Leu	Asp	Glu	Leu	Ala	Leu	Trp	Arg	Glu	Leu	Gly				
245				250				255											
Tyr	Thr	Ala	Arg	Ala	Pro	Arg	Phe	Ala	Ile	Ala	Tyr	Lys	Phe	Pro	Ala				
260				265				270											
Glu	Glu	Lys	Glu	Thr	Arg	Leu	Leu	Asp	Val	Ala	Phe	Gln	Val	Gly	Arg				
275				280				285											
Thr	Gly	Arg	Val	Thr	Pro	Val	Gly	Ile	Leu	Glu	Pro	Val	Phe	Leu	Glu				
290				295				300											
Gly	Ser	Glu	Val	Ser	Arg	Val	Thr	Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu				
305				310				315				320							

Glu	Leu	Asp	Ile	Arg	Ile	Gly	Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly
325				330				335							
Gly	Val	Ile	Pro	Glu	Val	Leu	Arg	Val	Leu	Lys	Glu	Arg	Arg	Thr	Gly
340				345				350							
Glu	Glu	Arg	Pro	Ile	Arg	Trp	Pro	Glu	Thr	Cys	Pro	Glu	Cys	Gly	His
355				360				365							
Arg	Leu	Leu	Lys	Glu	Gly	Lys	Val	His	Arg	Cys	Pro	Asn	Pro	Leu	Cys
370				375				380							
Pro	Ala	Lys	Arg	Phe	Glu	Ala	Ile	Arg	His	Phe	Pro	Ser	Arg	Lys	Ala
385				390				395				400			
Met	Asp	Ile	Gln	Gly	Leu	Gly	Glu	Lys	Leu	Ile	Glu	Arg	Leu	Leu	Glu
405				410				415							
Lys	Gly	Leu	Val	Lys	Asp	Val	Ala	Asp	Leu	Tyr	Arg	Leu	Arg	Lys	Glu
420				425				430							
Asp	Leu	Val	Gly	Leu	Glu	Arg	Met	Gly	Glu	Lys	Ser	Ala	Gln	Asn	Leu
435				440				445							
Leu	Arg	Gln	Ile	Glu	Glu	Ser	Lys	Arg	Arg	Gly	Leu	Glu	Arg	Leu	Leu
450				455				460							
Tyr	Ala	Leu	Gly	Leu	Pro	Gly	Val	Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu
465				470				475				480			
Ala	Ala	Arg	Phe	Gly	Asn	Met	Asp	Arg	Leu	Leu	Glu	Ala	Ser	Leu	Glu
485				490				495							
Glu	Leu	Leu	Glu	Val	Glu	Glu	Val	Gly	Glu	Leu	Thr	Ala	Arg	Ala	Ile
500				505				510							
Leu	Glu	Thr	Leu	Lys	Asp	Pro	Ala	Phe	Arg	Asp	Leu	Val	Arg	Arg	Leu
515				520				525							
Lys	Glu	Ala	Gly	Val	Glu	Met	Glu	Ala	Lys	Glu	Lys	Gly	Gly	Glu	Ala
530				535				540							
Leu	Lys	Gly	Leu	Thr	Phe	Val	Ile	Thr	Gly	Glu	Leu	Ser			
545				550				555				;			

AF092865 for *Thermus filiformis* Tok4A2 (SEQ. ID. No. 27), as follows:

Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly	Ala	Arg	Pro	Leu	Glu	Pro	Thr	Phe
1				5					10					15	
Arg	Pro	Val	Arg	His	Pro	Thr	Arg	Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe
20				25				30							

Thr	Tyr	Glu	Glu	Val	Leu	Ala	Phe	Glu	Glu	Arg	Leu	Asp	Arg	Ala	Leu
35				40				45							
Gly	Arg	Lys	Arg	Pro	Phe	Leu	Tyr	Thr	Val	Glu	His	Lys	Val	Asp	Gly
50				55				60							
Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu	Glu	Gly	Val	Leu	Val	Phe	Gly	Ala
65				70				75				80			
Thr	Arg	Gly	Asp	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
85				90				95							
Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg	Leu	Lys	Gly	Val	Pro	Asp	Arg	Leu
100				105				110							
Glu	Val	Arg	Gly	Glu	Val	Tyr	Met	Pro	Ile	Glu	Ala	Phe	Leu	Arg	Leu
115				120				125							
Asn	Glu	Glu	Leu	Glu	Glu	Arg	Gly	Glu	Lys	Val	Phe	Lys	Asn	Pro	Arg
130				135				140							
Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg	Gln	Lys	Asp	Pro	Arg	Val	Thr	Ala
145				150				155				160			
Lys	Arg	Gly	Leu	Arg	Ala	Thr	Phe	Tyr	Ala	Leu	Gly	Leu	Gly	Leu	Glu
165				170				175							
Glu	Ser	Gly	Leu	Lys	Ser	Gln	Tyr	Glu	Leu	Leu	Leu	Trp	Leu	Lys	Glu
180				185				190							
Lys	Gly	Phe	Pro	Val	Glu	His	Gly	Tyr	Glu	Lys	Ala	Leu	Gly	Ala	Glu
195				200				205							
Gly	Val	Glu	Glu	Val	Tyr	Gln	Ala	Xaa	Xaa	Xaa	Lys	Arg	His	Ala	Leu
210				215				220							
Pro	Phe	Glu	Ala	Asp	Gly	Val	Val	Val	Lys	Met	Asp	Asp	Leu	Thr	Leu
225				230				235				240			
Trp	Gly	Glu	Leu	Gly	Tyr	Thr	Ala	Arg	Ala	Pro	Arg	Phe	Ala	Ile	Ala
245				250				255							
Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Asn	Glu	Thr	Arg	Leu	Leu	Asp	Val	Asp
260				265				270							
Phe	Gln	Val	Gly	Arg	Thr	Gly	Arg	Val	Thr	Pro	Val	Gly	Ile	Leu	Glu
275				280				285							
Pro	Val	Phe	Leu	Glu	Gly	Ser	Glu	Val	Ser	Arg	Val	Thr	Leu	His	Asn
290				295				300							
Glu	Ser	Tyr	Ile	Glu	Glu	Leu	Asp	Ile	Arg	Ile	Gly	Asp	Trp	Val	Leu
305				310				315				320			

Val	His	Lys	Ala	Gly	Gly	Val	Ile	Pro	Glu	Val	Leu	Arg	Val	Leu	Lys	
				325					330					335		
Glu	Arg	Arg	Thr	Gly	Glu	Glu	Arg	Pro	Ile	Arg	Trp	Pro	Glu	Thr	Cys	
				340					345					350		
Pro	Glu	Cys	Gly	His	Arg	Leu	Leu	Lys	Glu	Gly	Lys	Val	His	Arg	Cys	
				355					360					365		
Pro	Asn	Pro	Leu	Cys	Pro	Ala	Lys	Arg	Phe	Glu	Ala	Ile	Arg	His	Phe	
				370					375					380		
Pro	Ser	Arg	Lys	Ala	Met	Asp	Ile	Gln	Gly	Leu	Gly	Glu	Lys	Leu	Ile	
				385					390					395	400	
Glu	Arg	Leu	Leu	Glu	Lys	Gly	Leu	Val	Lys	Asp	Val	Ala	Asp	Leu	Tyr	
				405					410					415		
Arg	Leu	Arg	Lys	Glu	Asp	Leu	Val	Gly	Leu	Glu	Arg	Met	Gly	Glu	Lys	
				420					425					430		
Ser	Ala	Gln	Asn	Leu	Leu	Arg	Gln	Ile	Glu	Glu	Ser	Lys	Arg	Arg	Gly	
				435					440					445		
Leu	Glu	Arg	Leu	Leu	Tyr	Ala	Leu	Gly	Leu	Pro	Gly	Val	Gly	Glu	Val	
				450					455					460		
Leu	Ala	Arg	Asn	Leu	Ala	Ala	Arg	Phe	Gly	Asn	Met	Asp	Arg	Leu	Leu	
				465					470					475	480	
Glu	Ala	Ser	Leu	Glu	Glu	Leu	Leu	Glu	Val	Glu	Glu	Val	Gly	Glu	Leu	
				485					490					495		
Thr	Ala	Arg	Ala	Ile	Leu	Glu	Thr	Leu	Lys	Asp	Pro	Ala	Phe	Arg	Asp	
				500					505					510		
Leu	Val	Arg	Arg	Leu	Lys	Glu	Ala	Gly	Val	Glu	Met	Glu	Ala	Lys	Glu	
				515					520					525		
Lys	Gly	Gly	Glu	Ala	Leu	Lys	Gly	Leu	Thr	Phe	Val	Ile	Thr	Gly	Glu	
				530					535					540		
Leu	Ser															
545	;															

AF092866 for *Thermus filiformis* Tok6A1 (SEQ. ID. No. 28), as follows:

Arg	Phe	Pro	Glu	Phe	Lys	Ser	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly
1	5				10				15						
Ala	Arg	Pro	Leu	Glu	Pro	Thr	Phe	Arg	Pro	Val	Arg	His	Pro	Thr	Arg
20				25				30							

Met	Tyr	Ser	Leu	Asp	Asn	Ala	Phe	Thr	Tyr	Glu	Glu	Val	Leu	Ala	Phe
35			40			45									
Glu	Glu	Arg	Leu	Glu	Arg	Ala	Leu	Gly	Arg	Lys	Arg	Pro	Phe	Leu	Tyr
50			55			60									
Thr	Val	Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu
65			70			75			80						
Glu	Gly	Val	Leu	Val	Phe	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Val	Gly
85			90			95									
Glu	Glu	Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg
100			105			110									
Leu	Lys	Gly	Val	Pro	Asp	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met
115			120			125									
Pro	Ile	Glu	Ala	Phe	Leu	Arg	Leu	Asn	Glu	Glu	Leu	Glu	Glu	Arg	Gly
130			135			140									
Glu	Lys	Val	Phe	Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg
145			150			155			160						
Gln	Lys	Asp	Pro	Arg	Val	Thr	Ala	Lys	Arg	Gly	Leu	Arg	Ala	Thr	Phe
165			170			175									
Tyr	Ala	Leu	Gly	Leu	Gly	Leu	Glu	Glu	Ser	Gly	Leu	Lys	Ser	Gln	Tyr
180			185			190									
Glu	Leu	Leu	Leu	Trp	Leu	Lys	Glu	Lys	Gly	Phe	Pro	Val	Glu	His	Gly
195			200			205									
Tyr	Glu	Lys	Ala	Leu	Gly	Ala	Glu	Gly	Val	Glu	Glu	Val	Tyr	Arg	Arg
210			215			220									
Phe	Leu	Ala	Gln	Arg	His	Ala	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	Val
225			230			235			240						
Val	Lys	Leu	Asp	Asp	Leu	Ala	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	Ala
245			250			255									
Arg	Ala	Pro	Arg	Phe	Ala	Leu	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Lys
260			265			270									
Glu	Thr	Arg	Leu	Leu	Asp	Val	Val	Phe	Gln	Val	Gly	Arg	Thr	Gly	Arg
275			280			285									
Val	Thr	Pro	Val	Gly	Val	Leu	Glu	Pro	Val	Phe	Ile	Glu	Gly	Ser	Glu
290			295			300									
Val	Ser	Arg	Val	Thr	Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu	Glu	Leu	Asp
305			310			315			320						
Ile	Arg	Ile	Gly	Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly	Gly	Val	Ile

325								330					335						
Pro	Glu	Val	Leu	Arg	Val	Leu	Lys	Glu	Arg	Arg	Thr	Gly	Glu	Glu	Arg				
340								345					350						
Pro	Ile	Arg	Trp	Pro	Glu	Thr	Cys	Pro	Glu	Cys	Gly	His	Arg	Leu	Val				
355								360					365						
Lys	Glu	Gly	Lys	Val	His	Arg	Cys	Pro	Asn	Pro	Leu	Cys	Pro	Ala	Lys				
370								375					380						
Arg	Phe	Glu	Ala	Ile	Arg	His	Tyr	Ala	Ser	Arg	Lys	Ala	Met	Asp	Ile				
385								390					395					400	
Glu	Gly	Leu	Gly	Glu	Lys	Leu	Ile	Glu	Arg	Leu	Leu	Glu	Lys	Gly	Leu				
405								410					415						
Val	Arg	Asp	Val	Ala	Asp	Leu	Tyr	His	Leu	Arg	Lys	Glu	Asp	Leu	Leu				
420								425					430						
Gly	Leu	Glu	Arg	Met	Gly	Glu	Lys	Ser	Ala	Gln	Asn	Leu	Leu	Arg	Gln				
435								440					445						
Ile	Glu	Glu	Ser	Lys	His	Arg	Gly	Leu	Glu	Arg	Leu	Leu	Tyr	Ala	Leu				
450								455					460						
Gly	Leu	Pro	Gly	Val	Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu	Ala	Arg	Arg				
465								470					475					480	
Phe	Gly	Thr	Met	Asp	Arg	Leu	Leu	Glu	Ala	Ser	Leu	Glu	Glu	Leu	Leu				
485								490					495						
Glu	Val	Glu	Glu	Val	Gly	Glu	Leu	Thr	Ala	Arg	Ala	Ile	Leu	Glu	Thr				
500								505					510						
Leu	Lys	Asp	Pro	Ala	Phe	Arg	Asp	Leu	Val	Arg	Arg	Leu	Lys	Glu	Ala				
515								520					525						
Gly	Val	Ser	Met	Glu	Ser	Lys	Glu	Glu											
530								535											

Arg	Ala	Leu	Gly	Arg	Lys	Gly	Pro	Phe	Val	Tyr	Thr	Val	Glu	His	Lys
50			55			60									
Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu	Glu	Gly	Ile	Leu	Val
65			70			75			80						
Trp	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Thr	Gly	Glu	Glu	Val	Thr	Gln
85			90			95									
Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg	Leu	Lys	Gly	Val	Pro
100			105			110									
Glu	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met	Pro	Ile	Glu	Ala	Phe
115			120			125									
Leu	Arg	Leu	Asn	Glu	Glu	Leu	Glu	Glu	Lys	Gly	Glu	Lys	Ile	Phe	Lys
130			135			140									
Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Phe	Arg	Gln	Lys	Asp	Pro	Arg
145			150			155			160						
Ile	Thr	Ala	Arg	Arg	Gly	Leu	Arg	Ala	Thr	Phe	Tyr	Ala	Leu	Gly	Leu
165			170			175									
Gly	Leu	Glu	Glu	Ser	Gly	Leu	Lys	Thr	Gln	Leu	Asp	Leu	Leu	His	Trp
180			185			190									
Leu	Arg	Glu	Lys	Gly	Phe	Pro	Val	Glu	His	Gly	Phe	Ala	Arg	Ala	Glu
195			200			205									
Gly	Ala	Glu	Gly	Val	Glu	Arg	Ile	Tyr	Gln	Gly	Trp	Leu	Lys	Glu	Arg
210			215			220									
Arg	Ser	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	Val	Val	Lys	Leu	Asp	Glu
225			230			235			240						
Leu	Ser	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	Ala	Arg	Ala	Pro	Arg	Phe
245			250			255									
Ala	Ile	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Lys	Glu	Thr	Ala	Leu	Phe
260			265			270									
Gln	Val	Val	Leu	Gln	Val	Gly	Arg	Thr	Gly	Gln	Val	Thr	Pro	Val	Gly
275			280			285									
Ile	Leu	Glu	Pro	Val	Phe	Ile	Glu	Gly	Ser	Glu	Val	Ser	Arg	Val	Thr
290			295			300									
Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu	Asp	Leu	Asp	Val	Arg	Ile	Gly	Glu
305			310			315			320						
Trp	Val	Leu	Val	His	Asn	Ala	Gly	Gly	Val	Ile	Pro	Glu	Val	Leu	Arg
325			330			335									
Val	Leu	Lys	Glu	Lys	Arg	Thr	Gly	Glu	Glu	Arg	Pro	Ile	Arg	Trp	Pro

340					345					350						
Glu	Thr	Cys	Pro	Glu	Cys	Gly	His	Arg	Leu	Val	Lys	Glu	Gly	Lys	Val	
355					360					365						
His	Arg	Cys	Pro	Asn	Pro	Leu	Cys	Pro	Ala	Lys	Arg	Phe	Glu	Ala	Ile	
370					375					380						
Arg	His	Tyr	Ala	Ser	Arg	Lys	Ala	Met	Asp	Ile	Gly	Gly	Leu	Gly	Glu	
385					390					395					400	
Lys	Leu	Ile	Glu	Lys	Leu	Leu	Glu	Lys	Gly	Leu	Val	Lys	Asp	Val	Ala	
405					410					415						
Asp	Leu	Tyr	Arg	Leu	Lys	Glu	Glu	Asp	Leu	Val	Gly	Leu	Glu	Arg	Met	
420					425					430						
Gly	Lys	Lys	Ser	Ala	Gln	Asn	Leu	Leu	Arg	Gln	Ile	Glu	Lys	Ser	Lys	
435					440					445						
Ala	Arg	Gly	Leu	Glu	Arg	Leu	Leu	Tyr	Ala	Leu	Gly	Leu	Pro	Gly	Val	
450					455					460						
Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu	Ala	Ala	His	Phe	Gly	Thr	Met	Asp	
465					470					475					480	
Arg	Leu	Leu	Glu	Ala	Ser	Leu	Glu	Glu	Leu	Leu	Gln	Val	Glu	Glu	Val	
485					490					495						
Gly	Glu	Leu	Thr	Ala	Arg	Gly	Ile	Tyr								
500					505;											

and AF092868 for *Tsp*. SM32 (SEQ. ID. No. 30), as follows:

Asp	Asn	Ala	Phe	Thr	His	His	Asp	Leu	Lys	Ala	Phe	Glu	Asp	Arg	Val
1				5					10					15	
Asp	Arg	Ala	Leu	Gly	Arg	Glu	Gly	Pro	Phe	Val	Tyr	Thr	Val	Glu	His
		20						25					30		
Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu	Glu	Gly	Ile	Leu
		35					40						45		
Val	Phe	Gly	Ala	Pro	Arg	Gly	Asp	Gly	Glu	Val	Gly	Glu	Glu	Val	Thr
	50					55					60				
Gln	Asn	Leu	Leu	Thr	Ile	Pro	Thr	Ile	Pro	Arg	Arg	Leu	Lys	Gly	Val
65					70					75					80
Pro	Glu	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met	Pro	Ile	Glu	Ala
				85					90					95	

Phe	Leu	Arg	Leu	Asn	Glu	Glu	Leu	Glu	Glu	Ala	Gly	Glu	Lys	Val	Phe	100	105	110
Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg	Gln	Lys	Asp	Pro	115	120	125
Arg	Ile	Thr	Ala	Lys	Arg	Gly	Leu	Arg	Ala	Thr	Phe	Tyr	Ala	Leu	Gly	130	135	140
Leu	Gly	Leu	Glu	Glu	Ser	Gly	Leu	Lys	Thr	Gln	Tyr	Glu	Phe	Leu	Leu	145	150	155
Trp	Phe	Lys	Glu	Lys	Gly	Phe	Pro	Val	Glu	His	Gly	Phe	Ala	Arg	Ala	165	170	175
Thr	Gly	Ala	Glu	Gly	Val	Glu	Arg	Val	Tyr	Gln	Gly	Trp	Leu	Gln	Lys	180	185	190
Arg	Arg	Lys	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	Val	Val	Lys	Leu	Asp	195	200	205
Glu	Leu	Ala	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	Ala	Arg	Ala	Pro	Arg	210	215	220
Phe	Ala	Ile	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Lys	Glu	Thr	Arg	Leu	225	230	235
Leu	Asp	Val	Val	Phe	Gln	Val	Gly	Arg	Thr	Gly	Arg	Val	Thr	Pro	Val	245	250	255
Gly	Ile	Leu	Glu	Pro	Val	Leu	Ile	Glu	Gly	Ser	Glu	Val	Ser	Arg	Val	260	265	270
Thr	Leu	His	Asn	Glu	Ser	Tyr	Ile	Glu	Glu	Leu	Asp	Ile	Arg	Ile	Gly	275	280	285
Asp	Trp	Val	Leu	Val	His	Lys	Ala	Gly	Gly	Val	Ile	Pro	Glu	Val	Leu	290	295	300
Arg	Val	Leu	Lys	Glu	Arg	Arg	Thr	Gly	Ala	Glu	Arg	Pro	Ile	Val	Trp	305	310	315
Pro	Glu	Asn	Cys	Pro	Glu	Cys	Gly	His	His	Leu	Val	Lys	Glu	Gly	Lys	325	330	335
Val	His	Arg	Cys	Pro	Asn	Pro	Leu	Cys	Pro	Ala	Lys	Arg	Phe	Glu	Ala	340	345	350
Ile	Arg	His	Tyr	Ala	Ser	Arg	Lys	Ala	Met	Asp	Ile	Gln	Gly	Leu	Gly	355	360	365
Glu	Lys	Leu	Ile	Glu	Lys	Leu	Leu	Glu	Asn	Gly	Leu	Val	Lys	Asp	Val	370	375	380

Ala	Asp	Leu	Tyr	Arg	Leu	Arg	Lys	Glu	Asp	Leu	Val	Gly	Leu	Glu	Arg
385					390					395					400

Met	Gly	Glu	Lys	Ser	Ala	Glu	Asn	Leu	Leu	Arg	Gln	Ile	Glu	Glu	Ser
				405					410					415	

Lys	His	Arg	Gly	Leu	Glu	Arg	Leu	Leu	Tyr	Ala	Leu	Gly	Leu	Pro	Gly
			420					425					430		

Val	Gly	Glu	Val	Leu	Ala	Arg	Asn	Leu	Ala	Ala	Arg	Phe	Gly	Thr	Met
		435					440						445		

Asp	Arg	Leu	Leu	Glu	Ala	Thr	Leu	Glu	Glu	Leu	Leu	Glu	Val	Glu	Glu
	450					455					460				

Val	Gly	Glu	Leu	Thr	Ala	Arg	Gly	Ile	Trp	Glu	Thr	Leu	Gln	Asp	Pro
465					470					475					480

Ala.

Please replace the paragraph beginning at page 10, line 25, with the following paragraph:

The thermostable ligase of the present invention is also characterized by having an arginine adjacent to the active site lysine (i.e. K) in the KXDG (SEQ. ID. No. 24) motif (where X is any amino acid).

Please replace the paragraph beginning at page 26, line 3, with the following paragraph:

The oligonucleotide perfect match substrate was formed by annealing two short oligonucleotides (33-mer for LP3'C (SEQ. ID. No. 11) and 30-mer for Com3F (SEQ. ID. No. 12)) with a 59-mer complementary oligonucleotide (Glg). Oligonucleotides LP3'C and Glg (SEQ. ID. No. 14) were in 1.5-fold excess so that all the 3' Fam labeled Com3F represented nicked substrates (see Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference). The T/G mismatch substrate was formed by annealing LP3'T (SEQ. ID. No. 13), which introduced a single base-pair mismatch at the 3'-end of the nick junction, along with Com 3'F to the complementary strand (Glg). The nicked DNA duplex substrates were formed by denaturing DNA probes at 94°C for 2 min followed by re-annealing at 65°C for 2 min in ligation buffer. The sequences of the oligonucleotides were listed below (p represents 5' phosphate group):

(SEQ. ID. No. 12) pAGTTGTCATAGTTTGATCCTCTAGTCTGGG-FAM-3' Com3
 LP3'T (SEQ. ID. No. 13) 5'- CCCTGTTCCAGCGTCTGCGGTGTTGCGTT
 LP3'C (SEQ. ID. No. 11) 5'-AAAACCTGTTCCAGCGTCTGCGGTGTTGCGTC
 Glg (SEQ. ID. No. 14) 3'-GGGACAAGGTTCGCAGACGCCACAACGCAGTCAACAGTATCAAAGTATCAAACTAGGAGATCAGACCC-5'

Please replace the paragraph beginning at page 27, line 15, with the following paragraph:

Amino acid sequence alignment of five Gram negative bacterial NAD⁺-dependent DNA ligases indicates that *Tth* ligase is 93% identical to *Thermus scotoductus* ligase, 49% to *Rhodothermus marinus* ligase, 48% to *E. coli* ligase, and 38% to *Zymomonas mobilis* based on sequence data retrieved from GeneBank. Degenerate primers corresponding to highly conserved regions of these ligases were used to amplify fragments of ligase genes from seven *Thermus* strains which represent a worldwide collection: *Thermus flavus* from Japan (SEQ. ID. No. 16), *Thermus aquaticus* YT-1 (SEQ. ID. No. 15) and *Thermus sp.* AK16D from Yellowstone National Park in the United States (SEQ. ID. No. 22), *Thermus filiformis* Tok4A2 (SEQ. ID. No. 17) and *Thermus filiformis* Tok6A1 (SEQ. ID. No. 18) from New Zealand, *Thermus sp.* SM32 (SEQ. ID. No. 19) from Azores, and *Thermus sp.* Vil3 (SEQ. ID. No. 20) from Portugal. The sequences of amplified ligase fragments ranging from 1.4 to 1.6 kb were determined by directly sequencing the PCR products using an ABI 373 automated sequencer. *Thermus* ligases, in general, were highly conserved during evolution as demonstrated by 85%-98% sequence identity. In contrast, the amino acid sequences of the restriction endonuclease *TaqI* and its isoschizomers from the identical strains show only 50-70% aa identities (Cao, et al., *Gene*, 197:205-214 (1997), which is hereby incorporated by reference). *Thermus* ligases in general show 30-40% sequence identities as compared with DNA ligases from other bacteria. The sequence divergence is slightly higher among the different geographic groups than within the same group, which may reflect random drift or adaptation to their respective local environments (Figure 1). *Thermus flavus*, *Thermus filiformis* Tok4A2, *Thermus filiformis* Tok6A1, *Thermus sp.* SM32, *Thermus sp.* Vil3, *Thermus aquaticus* YT-1, and *Thermus sp.* AK16D (SEQ. ID. No. 14-20) ligases shared 98.2%, 89.9%, 89.5%, 89.8%, 88.3%, 88.2%, 88.1% with *Thermus thermophilus* HB8 DNA ligase, respectively. The adenylation site of the enzymes (¹¹⁸KXDG (SEQ. ID. No. 24) where X is in general a hydrophobic residue), as identified by site-directed mutagenesis of *Tth* DNA ligase, is completely identical among all *Thermus* ligases, furthermore, the flanking sequences of the adenylation motif are also identical except *Tsp.* AK16D in which the aa residues ¹¹⁷H before the ¹¹⁸K is substituted by an ¹¹⁸R ¹¹⁷R (Figure 1B). In non-*Thermus*

NAD⁺-dependent ligases discovered to date, the corresponding position is either a Pro or a Leu. The two isolates from Japan can be distinguished from the other *Thermus* strains by a 3-aa-insertion at position 234.

Please replace the paragraph beginning at page 29, line 24, with the following paragraph:

Divalent metal ion is indispensable for each of the three steps in a ligation reaction: (i) adenylation of a lysine residue in the adenylation motif KXDG (SEQ. ID. No. 24); (ii) transfer of the adenylate to the 5' phosphate to form a DNA-adenylate intermediate; and (iii) formation of the phosphodiester bond with the release of adenosine monophosphate (AMP). In general, Mg²⁺ is the preferred metal ion for both ATP-dependent and NAD⁺-dependent ligases. Mg²⁺ was substituted with alkaline earth metal ion Ca²⁺ and commonly studied period 4 transition metal ions. *Tth* and *Tsp*. AK16D ligases could use Mn²⁺ as an alternative metal cofactor to support ligation activity (Figure 4). Both enzymes were less active with Ca²⁺, while Co²⁺, Ni²⁺, Cu²⁺, and Zn²⁺ failed to support ligation. In comparison, ATP-dependent ligase from *Hin* (i.e. *Haemophilus influenzae*) uses only Mg²⁺ and Mn²⁺ as the metal cofactor for nick closure but not Ca²⁺, Co²⁺, Cu²⁺, and Zn²⁺ (Cheng, et al., Nucleic Acids Res, 25(7):1369-1374 (1997), which is hereby incorporated by reference); ATP-dependent ligase from *Chlorella* virus PBCV-1 can use Mg²⁺, Mn²⁺, and Co²⁺ but not Ca²⁺, Cu²⁺, and Zn²⁺ (Ho, et al., J Virol, 71(3):1931-1937 (1997), which is hereby incorporated by reference). Using Ca²⁺ as the metal cofactor, *Thermus* enzymes were able to convert most of the substrate into the DNA-adenylate intermediate. However, the rates of nick closure were reduced which led to the accumulation of the DNA-adenylate intermediate (Figure 4B). A small amount of the intermediate was observed with Ni²⁺; however, ligation product was not observed at the current detection level, suggesting that Ni²⁺ could not support the nick closure step (Figure 4B). To further compare the relative activity of the two *Thermus* ligases with Mg²⁺ and Mn²⁺, the generation of ligation product was first monitored over a 20-min time period. As shown in Figure 5, the *Thermus* enzymes were consistently more active with Mg²⁺ than with Mn²⁺. Second, ligation activity up to 40 mM Mg²⁺ or Mn²⁺ concentrations (Figure 6) was assayed. Both of the enzymes responded sensitively to the change of the metal ion concentration in the reaction mixture. At high M²⁺ concentrations, the high ionic strength may inhibit the enzyme activity, consistent with KCl dependence profile (Figure 4).

Similar to the time-course results, the *Thermus* enzymes were more active with Mg^{2+} than with Mn^{2+} (Figure 6). The discrepancy on the relative activity of *Thermus* ligases between this study and an earlier report may be due to use here of cloned enzymes while the earlier work used purified native enzyme (Takahashi, et al., J Biol Chem, 259(16):10041-10047 (1984), which is hereby incorporated by reference).

Please replace the paragraph beginning at page 35, line 24, with the following paragraph:

Studies on *Tth* DNA ligase has deepened understanding of thermostable ligases and has reaffirmed the common theme of ligation — adenylation of ligase at the KXDG (SEQ. ID. No. 24) motif (Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996), which is hereby incorporated by reference). This study reveals that *Thermus* ligases may differ from each other as to substrate specificity despite their highly identical primary protein sequences. A highly homologous structure can be anticipated from various *Thermus* ligases, but subtle local environments may dictate the probability of accepting a particular mismatch as the substrate. The fidelity of the *Thermus* ligases may be determined by multiple domains, multiple motifs and/or multiple sequence elements. In comparison of *Tth* and *Tsp*. AK16D ligases, one can find that although K294R (in an identical local environment, see Figure 1B) enhances the fidelity of *Tth* ligase (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference), *Tsp*. AK16D ligase with a K in this position can still demonstrate superior mismatch discrimination. Additional sequence elements remain to be uncovered. The R substitution at the adjacent position to the KXDG (SEQ. ID. No. 24) motif may have an effect on the *Tsp*. AK16D ligase's specificity, because studies on *Chlorella* ligase has emphasized the importance of occupying AMP binding pocket for nick recognition (Sriskanda, et al., Nucleic Acids Res, 26(2):525-531 (1998)). The accumulation of DNA-adenylate intermediate with some divalent metal ions by *Tsp*. AK16D ligase asserts that the nick closure step of a ligation reaction can be sensitive to the selection of metal ions, gapped substrates and mismatch substrates. More structural and functional studies on *Tsp*. AK16D ligase could reveal how this enzyme achieves high fidelity with different substrates and different metal ions.